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ORGANIZATION OF THE TELIAL SORUS IN THE PINE RUST, GALLOWAYA PINICOLA ARTH [1]

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INTRODUCTION

The short-cycled pine rust now commonly referred to as Gallowaya (*2*)[2] was first reported under the name *Coleosporium pini* by Galloway (*9*), who later (*10*) described its effects on the host *Pinus virginiana*. Although he was not fully aware of the exact nature of the germination of the teleutospores, he figured in some detail various stages in the development of the elements of the sorus and brought out some of the most characteristic features of the fungus. The writer was enabled to make a further study of the fungus from material furnished by W. W. Diehl, who collected quantities of the rust for him in the vicinity of Washington, D. C. It will be shown that there is formed a distinct and persistent peridial buffer structure which functions in rupturing the leaf tissues overlying the young sorus and that, following cell fusions, teleutospores are borne in chains. The spores are not sessile in the sense that only one spore is cut off from a basal cell as in Coleosporium. Neither does the basal cell bud to form the spores as in Puccinia.

THE GAMETOPHYTIC ELEMENTS

As no one had questioned the results of Galloway's infection work in demonstrating that the rust is short-cycled, it was to be expected that the cells of the mycelium in the pine leaf would be uninucleated. This is clearly the case. The hyphae are readily stained, although the waxy nature of the young sorus renders it difficult always to get good fixation of later stages. Each cell contains a single nucleus (fig. 1, B).

Vestigial spermogonia are to be found occasionally, but the host tissues above them (pl. 1, A) are not fully ruptured and spermatia are seldom formed. The few spermogonia seen in the sections were located beneath stomata. This may be of no significance, as they are rather broad and the stomata are scattered along parallel lines on the flat side of the leaf. The weft of hyphae just below the base of the primordium is a little denser than that which is to produce a telium. The end cells which are somewhat enlarged send out smaller branches which converge slightly so that they appear to be directed toward the stomatal opening. Galloway's Figure 10 (*10*) might very well have been drawn from a section of a spermogonium.

[1] Received for publication Nov. 13, 1924; issued December, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 651.

FIG. 1.—A, young primordium before cell fusions; B, first cell fusions, two sterile cells above; C, cell fusion, three sterile cells above; D, first mature spore; E, margin of sorus, fusions at different levels; F, chains of young spores, "peridium" (*a*) above; G, oblique cell divisions; H, I, basidia supported by elongated part of cell wall (*b*); J, K, spore chains in old sorus, stalklike remains of spore above; L, typical spore chain; M, N, basidial cells with additional body in each near nucleus. (C, L, and N are more highly magnified, and M and E less highly magnified, than the others.)

THE PERIDIAL BUFFER TISSUE

There is very little intermingling or intertwining of hyphae as a preliminary to the formation of the telial sorus. The hyphal complex below is usually no denser than it is in other parts of the leaf. The hyphae push out between the mesophyll cells and widen suddenly just before reaching the ends of the large lobes of these cells. Since the hyphal branches grow straight out against the hypodermal tissue in a solid rank, the epidermis and underlying tissue are stretched and pushed outward. Each chain in the palisade of fungus tissue is composed of four or five uninucleated cells (fig. 1, A). The buffer effect is considerably increased as the terminal cells lose their granular cytoplasmic contents and elongate (fig. 1, C, *a*). The propriety of calling the layer of terminal cells which persists a peridium is discussed later.

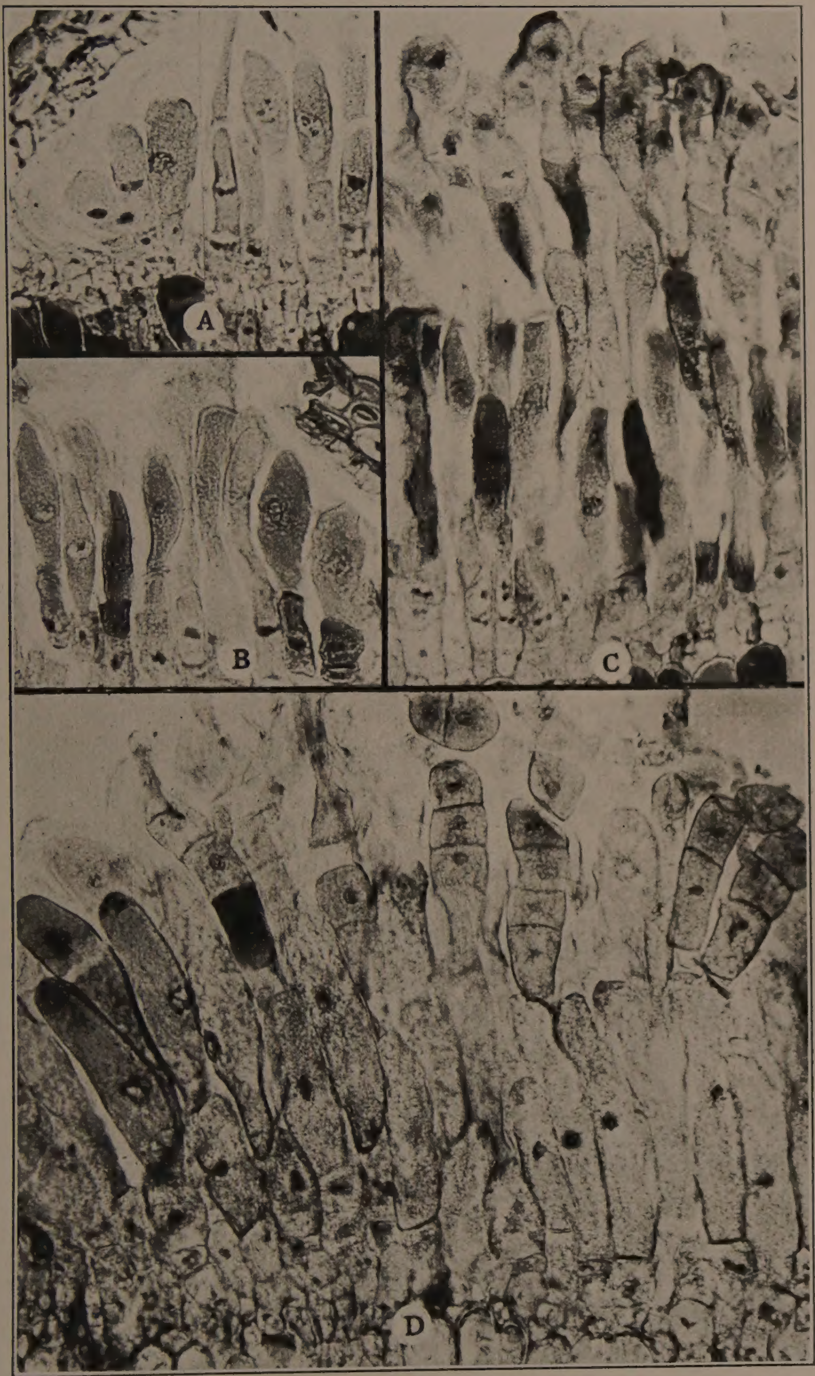
CELL FUSIONS

Up to this time there is nothing in the appearance of the cells of the primordium to indicate exactly where cell fusions are to occur. Soon after the epidermis is ruptured, however, the sorus takes on quite a different appearance. Cell fusions which are readily distinguished are seen along a line beneath the point of rupture (fig. 1, B to D). The fusing cells are the second or third cells from the outer ends of the chains, and are therefore intercalary. The writer has not seen fusions between subterminal cells, but this may sometimes occur. Neither are the fusions all on the same level (fig. 1, E). There are more frequently two sterile cells in the chains above the fusing cells (fig. 1, B and D). The conjugations are more readily seen in sections that show the gaps between the lobes of the mesophyll cells. The adjacent walls of the fusing cells quickly disappear, and the two nuclei come closer together (fig. 1, C). The cell thus formed enlarges but its cytoplasm becomes only slightly less vacuolate. At the same time the buffer cells immediately above collapse. The terminal cells of the chains, however, retain their form and persist indefinitely as a sort of peridium, or until cast off with fragments of the ruptured leaf tissue above (pl. 1, C).

CATENULATE SPORES

The fusion cell quickly divides, and the upper daughter cell develops a very dense cytoplasm so that it takes the stains readily. The fusion cell still remains vacuolate. As the sorus ages and increases in size, the buffer cells at the margin elongate correspondingly. The digestive effect which the newly formed spore cells have on this tissue is strikingly demonstrated by the way the fertile layer broadens laterally (pl. 2, A, and fig. 1, E). Although the subterminal cells in the chains are completely destroyed, the terminal cells, at least the outer ends of them, persist, as noted above (pl. 1, C). From this time on, the number of binucleated spore cells cut off in the chain above each fusion cell increases. At the margin, nuclear fusions may occur in the second or third spore of the chain (fig. 1, E); while at the center, there may exist three or four binucleated potential spore cells, above which there are one or two spores, considerably elongated and rich in cytoplasm, but still binucleated (fig. 1, K). Above these the spores are much longer and uninucleated (fig 1, J

A.—Spermogonium of Gallowaya
B.—Telial sorus before cell fusions
C.—Sorus showing mature spores, and remains of peridial buffer tissue
(Same magnification in the three figures)



A.—Chains of spores and "peridium" at margin of a sorus
B.—Chains of spores in a young sorus
C.—Spores with stalk-like lower ends, in an old sorus
D.—Spore chains terminated by mature basidia
(Same magnification as in Plate 1)

to L). It would seem that there can be no question that the spores of Gallowaya are catenulate, since it is possible, in a sorus sufficiently developed, to trace the chain upward from the "two-legged" fusion cell below to the germinated spore above (fig. 1, I and L). Ordinarily, then, one could say that at the middle of the mature sorus the chain of cells may be composed of a fusion basal cell; two to four short vacuolated binucleated cells; one or two elongated binucleated spores rich in cytoplasm; one or two long uninucleated spores; and the terminal germinated spore in the form of a four-celled internal basidium (fig. 1, L).

INTERCALARY ELEMENTS

A feature which is brought out more or less diagrammatically in Figure 1, L, *b*, is well shown in Plate 2, D. In some of the chains of cells there is nothing to suggest that these intercalary structures are cells. At H, however, in Figure 1, *d* is a cell which is clearly degenerating, so that one might think that both *b* and *d* are real intercalary cells. C. R. Orton has suggested to the writer that if such were the regular order of development throughout, it might be concluded that the sorus in Gallowaya is a modified aecidium. Such a form would round out an evolutionary series nicely. Until further evidence is found, however, the writer is compelled to consider this intercalary structure, *b*, as formed merely by the elongation of the lower end of the lower cell of the basidium. The wall seems to become separated from the cytoplasmic contents above and elongates in the form of a stalk, pushing the basidium still farther outward. In an aecidium, for example, when the spore is formed, its sister cell is usually sacrificed to serve, as some hold, merely as a disjunctor, or, as others might claim, to make available sufficient food for the maturing of one of the pair. Where the upper daughter cell degenerates and the lower one becomes a spore, the first of these views could not be held. In some cases the intercalary cell elongates to perform the function of a stalk. In Gallowaya, some of the true spores fail to develop, and they may then elongate greatly and function as stalk elements. This intercalary portion seems to be simply the swollen end of the cell wall.

Just how many of the cells in the chain function as spores is problematical. Typically, at any given time only the terminal ones do germinate. After the discharge of the sporidia the walls of the basidium collapse and disappear. Following this there is a sudden stretching or elongation at the lower end of the next spore. There appears to be a limit to the amount of elongation this spore can undergo before processes of disorganization begin. The next spore below, by elongating also, helps to bring the new terminal spore into a favorable position as regards its competitors (fig. 1, I). There is no question that as the sorus ages the lower spores in the chains never succeed in functioning as spores. They are, however, called upon to serve a very useful purpose, for it is only by their sacrifice in excessive elongation that the spore above can be brought into a position favorable for the discharge of its sporidia (fig. 1. I to K). This sacrifice would not be necessary if young spores were being continuously cut off from the basal cell below. In an old sorus, therefore, one frequently finds germinated spores connected directly with the binu-

cleated cells at the base of the sorus by means of a granular aggregation of decomposition products (fig. 1, I) stretched out so that it simulates the expanded stalk of a spore of Gymnosporangium. At different levels on all sides, uninucleated spores are still present (pl. 2, C). Such a picture is misleading, for it suggests that all of the spores are at first sessile, but that by a stretching of the basal part, and as a result of lateral pressures, the spores are brought to different levels.

The spores developed at the margin of the sorus are greatly deformed and truncated (pl. 1, C). Though the terminal ones do germinate to form their internal basidia (fig. 1, H) they can be brought out into the open only by the great elongation of the spore below. Cross walls are not always laid down at right angles to the spore axis. This gives the spore cells the appearance of budding out at the side (fig. 1, G), especially if the wall of the spore above separates from the spore contents and undergoes mucilaginous disorganization.

Each cell of the internal promycelium has a single nucleus. One occasionally finds what might be taken to be two nuclei in each cell (fig. 1, M, N). One of the bodies, which is clearly a nucleus, is larger than the other. The smaller body seems to be more uniform in its composition, and stains less like a nucleus. Good fixation of these stages was not obtained. Binucleated sporidia are often found in the rusts, and it is possible that the original nucleus of the basidial cell sometimes divides precociously instead of waiting until it has passed up into the sporidium.

DISCUSSION

The amount of sterile tissue which is found overlying the sporogenous cells in a rust sorus may not now depend altogether on the depth at which the primordium develops, but it is generally conceded that the location of a sorus with respect to the host tissues did, in evolution, have a considerable influence in determining the type of primordium evolved. Arthur (*2*) and others early recognized the importance of this feature. In some forms of deep-seated aecidia, the primordium is composed of a mass of intertwined hyphae without orientation. The host tissues are crowded aside and, by subsequent disorganization of the fungus tissue at the center, a cavity is developed and food made available for the growth of spores. Where a resistant host tissue above is to be ruptured, one may find that the sorus primordium is composed of a palisade arrangement of hyphal cells thrust against the tissue to be broken away.

Had Lindfors (*13*) studied the organization of the telial sorus in Gymnosporangium and Cronartium and the uredinium in Pucciniastrum and Cronartium, or the deep-seated aecidia in certain Peridermiums, he would no doubt have anticipated the objections that have been raised against the theory that the sterile cells above the fusing cells are the morphological representatives of ancestral red-alga trichogynes, a theory which the propounder himself would no longer support. The nature of the sterile tissue lying above the fertile or fusing cells in a sorus is best understood, not by limiting one's studies of rusts to cell fusions, but by also investigating those sori where no fusions occur. Colley's method of study (*4*) of the white-pine blister rust, *Cronartium ribicola*, is to be commended particularly on this account.

As the writer interprets Colley's discussion of the development of the uredo sorus in *Cronartium ribicola*, the "peridium" is the exact homologue of the buffer tissue in Gymnosporangium. Certain cells of the uredo primordium become oriented vertically, and, by elongating, develop a thrust against the epidermis. These cells divide, and the upper daughter cells, in conjunction with their homologous neighbors, constitute the peridium made up of elongated cells. The subterminal cells now divide to give rise to spore initials above and basal cells below. The telial peridium is evidently developed in much the same way.

It has been found (*5*) that in several species of Gymnosporangium the teleutospores arise from the subterminal cells of the chains composing the primordium. The terminal cells, by swelling and elongating, serve as a buffer tissue to break open the overlying host tissues. The sacrifice of these terminal cells is made necessary by the fact that the tissues above the sorus are very tough, even in the case of leaf sori. The substance originally contained in the cells of the buffer tissue is not wasted, however. The teleutospore buds, by growing up through or between the sterile cells, absorb their predigested disorganization products, just as in all sorts of fungi one sees cases of self-parasitism in the form of "Durchwachsung" phenomena.

In the uredo sorus of Pucciniastrum (*12, 6*) the terminal cells of the sorus primordium are sterile and, though persisting as a "peridium," first lose their contents, swell and elongate, and function primarily as a buffer tissue. Adams (*1*), in a paper on the Peridermiums, shows that the fusing cells are merely intercalary cells in a space making buffer complex of parallel hyphae. In deep-seated sori there may be a half dozen or more cells above the fusing cells in the chain. The writer has found no evidence anywhere in the rusts that these chains are multicellular trichogynes.

Even though a buffer tissue persists more or less, as it does in Gallowaya, it should not be confused with a peridium. Morphologically, the latter structure in the rusts, if one is to take the peridium of the aecidium as the standard, is composed of spore and intercalary cells. In the uredo sorus of Pucciniastrum (*6*) the buffer tissue may be a true peridium, unless Kursanov (*12*) was correct in his statement as to this structure in *Pucciniastrum pirolae*. He claims that the intercalary cells which lie immediately below the terminal cells are cut off by the cells below. This would make the "peridium" of Pucciniastrum the homologue of the "peridium" of the uredinium of Cronartium and of the telium of Gymnosporangium, in neither of which is the structure a true peridium.

There has recently been described (*8*) a short-cycled strain of *Caeoma nitens* in which no spermogonia are developed. In every such case the aecidiospore arising without cell fusion is uninucleated, and on germination produces a two-celled promycelium with only two sporidia. The fact that Gallawaya develops at most only vestigial spermogonia does not seem to be of any particular effect on future growth processes. The internal promycelium is four-celled and produces at least four sporidia. Lindfors (*13*) says that in *Puccinia arenariae*, a short-cycled rust, the teleutospore produces a two-celled promycelium. Cell fusions do not occur in the sorus, the cells of the mycelium being already binucleated. A nuclear fusion in the spores is followed by two divisions, but the septa which divide the

structure into four cells are not laid down. There are two nuclei in each of the cells of the two-celled promycelium, and two nuclei are supposed to enter each sporidium. Lindfors shows two teleutospores germinating with long germ tubes, and he states that Grove had found four-celled promycelia in this species. Evidently this species of rust must receive further attention before it can be compared with the strain of the short-cycled *Caeoma nitens* which develops two-celled promycelia.

In any system of classification based on morphology it may be asked just how important, as showing relationship, is the manner of germination of a spore. Grove (*11*) says, "It is, indeed, doubtful whether the character upon which the Coleosporiacae are united into one group, viz, the internal basidium, is really an indication of close affinity."

If the spores of *Chrysomyxa abietis*, which according to Kursanov (*12*) and Lindfors (*13*) are borne in chains, germinated with internal basidia instead of as they do, would this species be placed in the genus Gallowaya and in the group with Coleosporium? Except for the proliferation of the binucleated cell formed after cell fusion in this Chrysomyxa, there is not very much difference between it and Gallowaya so far as the organization of the sorus is concerned. In both cases the fusing cells are topped by two or three sterile cells. A short chain of binucleated cells is developed after cell fusion. These cells clearly never function as spores; they remain binucleated to the end, representing a short sporophytic generation. Nuclear fusion occurs in the outermost cells, so that a chain of uninucleated teleutospores is formed. Lindfors (*13*) doubts very much whether all of the uninucleated spores function in this Chrysomyxa, since in some cases the lower ones seem to lose most of their cytoplasm and become elongated. This is what often occurs in Gallowaya. In discussions of morphology it is not a question whether a structure functions, or whether a spore germinates, or what form the germ tube takes, but it should be asked, What are its phylogenetic antecedents?

Regardless of the merits of Grove's contention that Ochropsora is not one of the Coleosporiaceae, there can be no question that a system of classification based on the manner of spore germination is not a natural one. The writer (*7*) has shown that in certain strains of *Caeoma nitens* some of the aecidiospores produce long germ tubes which reinfect the host locally so that eventually teleutospores appear in the life cycle, while other aecidiospores, even from the same sorus, produce promycelia directly. So long as an unstable condition exists, such strains of orange-rust of Rubus can not be separated generically.

Galloway (*10, p. 443*) says: "The entire contents of the cell seem to be used up in the formation of the promycelium (sterigma) and the sporidium, and if this is not the case, the formation of secondary sporidia goes on until there is no protoplasm left." W. W. Diehl has informed the writer that he has seen cases where more than one sporidium was formed on the cell outgrowth in Gallowaya. Possibly in rare cases a true external promycelium is formed in Gallowaya. The writer has not seen young spores of this form with more than two nuclei. After germination, however, one can frequently find basidial cells with two bodies, as noted previously, both

very similar to nuclei and lying close together. If two sporidia are to be formed in succession from a single cell, one nucleus must remain behind after the first sporidium has been supplied. So far, this process has not been demonstrated for the rusts, although it is well known in the smuts. Even though two or more sporidia should be formed on the outgrowth from the same cell in Gallowaya, that outgrowth can not be called a promycelium, nor the cell from which it arises a teleutospore. Cells of teleutospores are mother cells, with which reduction divisions are normally associated. In this genus there is a series of binucleated "sporophytic" cells cut off from a fusion cell. Beginning with the oldest cell, the two nuclei fuse in regular order. The fusion nucleus in the oldest spore divides twice, a four-celled structure replacing the uninucleated cell. This may be sufficient evidence for assuming that the binucleated cells below are young spores and the uninucleated cells above them are certainly mature spores. Each four-celled structure is a protobasidium or internal promycelium.

In characterizing Coleosporium, Arthur (*3*), on the basis of what was then known of the telial sorus, says: "Teliospores sessile (by successive formation and displacement due to lateral pressure often appearing catenulate and pedicellate) * * *. By "successive formation" is evidently meant either that a new basal cell displaces the one from which a spore has been cut off, or the original basal cell buds out from the side to form a new spore. It is difficult to understand how the former process could take place once the primordium was organized. As far as the writer is aware, no one has yet reported a Coleosporium in which the basal cell buds out to form a number of spores. It is certain that such methods of spore formation do not occur in Gallowaya. The pedicellate appearance of its spores is in part due to displacement and lateral pressure, but its spores are nevertheless borne in chains. If the more or less persistent sterile tissue. enveloping the uredo sori in Hyalopsora, Pucciniastrum, and Cronartium can be called a peridium, then the similar structure in Gallowaya must be referred to by the same designation.

SUMMARY

The mycelial hyphae of *Gallowaya pinicola* are composed of uninucleated cells. Aborted or vestigial spermogonia are sometimes developed between the mesophyll and the overlying hypodermal tissue. Spermatia are seldom formed.

The telial primordium first develops a buffer tissue composed of chains of cells, the terminal ones swelling or elongating somewhat, thus breaking open the overlying host tissues.

Cell fusions occur between intercalary cells in the chains composing the primordium. The fusing cells are the third or fourth cells from the ends.

Several binucleated cells are cut off above the fusion basal cell. These are at least potentially spores. Nuclear fusions occur in regular order, beginning with the oldest binucleated cell. As the terminal spore germinates, the lower end of its cell wall swells and elongates, thus thrusting the protobasidium still farther out in the sorus. Not all of the cells in a chain necessarily function as spores. Some of them become disorganized, owing to excessive elongation.

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